



CERTIFICATION

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Certificate No.

021401

The AOAC Research Institute hereby certifies the method known as:

Easy Plate CC

manufactured by

**Kikkoman Biochemifa Company
2-1-1, Nishi-shinbashi
Minato-ku, Tokyo 105-0003
Japan**

This method has been evaluated in the AOAC® *Performance Tested Methods*™ Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC® Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*™ certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above-mentioned method for a period of one calendar year from the date of this certificate (December 08, 2021 – December 31, 2022). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

December 08, 2021

Date

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KIT NAME(S) Easy Plate CC Formerly known as Medi-Ca CC	CATALOG NUMBERS 61981	
INDEPENDENT LABORATORY Q Laboratories, Inc 1400 Harrison Avenue Cincinnati, OH 45214 USA	AOAC EXPERTS AND PEER REVIEWERS Yi Chen ¹ , Yvonne Salfinger ² , Wayne Ziemer ³ ¹ US FDA, CFSAN, College Park, MD, USA ² Consultant, Denver, CO USA ³ Consultant, Loganville, GA, USA February 2020 modification reviewed internally	
APPLICABILITY OF METHOD Target organism – Coliform bacteria	REFERENCE METHOD <i>Bacteriological Analytical Manual</i> , Chapter 4, Section G) (3)	
Matrixes – (50 g) - raw ground pork, raw lamb, raw ground chicken, raw tuna fillet, raw salmon fillet, raw shrimp, fresh peeled banana, fresh cut pineapple, and fresh cut apples		
Performance claims - The method is a reasonable alternative to the Violet Red Bile Agar method (<i>Bacteriological Analytical Manual</i> , Chapter 4, Section G) (3) for raw meat, raw poultry, raw fish and fresh fruits.		
ORIGINAL CERTIFICATION DATE February 03, 2014	CERTIFICATION RENEWAL RECORD Renewed annually through December 2022.	
METHOD MODIFICATION RECORD 1. February 2020 Level 2 2. November 2020 Level 1 3. June 2021 Level 1	SUMMARY OF MODIFICATION 1. Manufacturing location change from Tokyo, Japan to Kanagawa, Japan. 2. Editorial and formatting changes to insert. 3. Rebranded kit to reflect Kikkoman and method name change from Medi-Ca CC to Easy Plate CC.	
Under this AOAC® <i>Performance Tested</i> ™ License Number, 021401 this method is distributed by: 1. AS ONE Corporation 2. KENIS LIMITED 3. Nippon Bacterial Test CO., LTD. 4. FUJIFILM Wako Pure Chemical Corporation 5. Microgiene Co. LTD 6. Weber Scientific 7. ELMEX Limited	Under this AOAC® <i>Performance Tested</i> ™ License Number, 021401 this method is distributed as: 1. Easy Plate CC 2. Easy Plate CC 3. Easy Plate CC 4. Easy Plate CC 5. Easy Plate CC 6. Easy Plate CC 7. Easy Plate CC	
PRINCIPLE OF THE METHOD (1) Easy Plate CC (formerly Medi-Ca CC) is a ready-made dry medium for coliform count made up of four components: a waterproof sheet, a dry medium containing a gelling agent and the chromogenic enzyme substrate, 5-Bromo-4-chloro-3-indolyl β-D galactopyranoside (X-gal), a hydrophobic resin ring surrounding the medium, and a transparent cover over the medium. Sample suspension is dispensed on the center of the medium while the cover is lifted. After that, the cover is dropped gently to spread the suspension on the medium evenly. The suspension rapidly soaks into the medium, which turns into a gel in 3 minutes. The incubation of the sheet at 35 ± 1°C for 24 ± 1 h develops blue colonies because of the enzymatic reaction involving the substrate: the β-galactosidase produced by bacteria catalyzes the hydrolysis of the X-gal to yield an insoluble blue product.		

DISCUSSION OF THE VALIDATION STUDY (1)

The Easy Plate CC method was compared to the VRBA method (BAM, Chapter 4, Section G) for the nine raw foods from the four food categories. The 95% CI for the mean difference between the two methods at each contamination level for seven matrixes from all four categories fell within the range of -0.50 to 0.50, and no statistical difference was observed at all three contamination levels for four matrixes from three categories (Table 2 and 5). In addition, the repeatability of the Easy Plate CC method was overall similar to that of the VRBA method. These results demonstrated that the Easy Plate CC method is a reasonable alternative to the VRBA method for raw meat, raw poultry, raw fish and fresh fruits.

In contrast to the internal lab study, the Easy Plate CC method produced the remarkably lower coliform count than the VRBA method at the high level for raw salmon fillet in the independent lab study (Table 5). The explanation for this is that non-coliform bacteria in large numbers competed with total coliform and made it difficult for coliforms to be detected: approximately 80% of the total Coliform colonies could not be recognized due to their tiny sizes or slight intensities. The Easy Plate CC method is subject to that kind of growth inhibition because a gel volume of a Easy Plate CC medium is roughly ten times smaller than an agar volume of VRBA. In fact, a total viable count of the sample stored in a storage condition in the independent lab study (at 2-5°C for 5 days) was ca. 10⁷ CFU/g, being one hundred times higher than that of the one stored in a storage condition in the internal lab study (at 10 ± 1°C for 24 ± 1 h). In addition, the third party pointed out that the low level had distinct dark blue colonies and the high level had small light blue colonies. Probably, psychrophiles such as *Pseudomonas* selectively grew to inhibit the growth of coliforms in the independent lab study. In that case, an additional incubation for a few hours for making the colony size larger or the colony color intensity darker is recommended to obtain the colony count results equivalent to the VRBA method.

Table 1. Inclusivity/Exclusivity Panel Results (1)

Strain Name	Source ^a	Origin	Coliforms		Results ^b	BGLB
			Medi-Ca CC	BGLB		
<i>Citrobacter koseri</i>	NBRC 105690	Unknown	+	+		
<i>Citrobacter amalonaticus</i>	NBRC 13547	Unknown	+	+		
<i>Citrobacter freundii</i>	ATCC 8090	Unknown	+	+		
<i>Citrobacter freundii</i>	NBRC	unknown	+	+		
<i>Citrobacter koseri</i>	Natural isolate #20	White radish sprouts	+	+		
<i>Cronobacter sakazakii</i>	NBRC 102416T	Child's throat	+	+		
<i>Cronobacter sakazakii</i>	NBRC 105698	Child's throat	+	+		
<i>Cronobacter sakazakii</i>	Natural isolate #6	Green soybeans	+	+		
<i>Enterobacter aerogenes</i>	NBRC 13534T	Sputum	+	+		
<i>Enterobacter aminigenus</i>	NBRC 105700T	soil	+	+		
<i>Enterobacter cloacae</i>	NBRC 13535T	Spinal fluid	+	+		
<i>Enterobacter cloacae</i>	NBRC 13536	Unknown	+	+		
<i>Enterobacter cloacae</i>	NBRC 12935	Unknown	+	+		
<i>Enterobacter cloacae</i>	NBRC 12937	Diseased silk-worm	+	+		
<i>Enterobacter cloacae</i>	ATCC 222	Unknown	+	+		
<i>Enterobacter cloacae</i>	Natural isolate #21	White radish sprouts	+	+		
<i>Enterobacter gergoviae</i>	NBRC 105706T	Urine	+	+		
<i>Escherichia blattae</i>	NBRC 105725T	Hindgut of cockroach	-	+		
<i>Escherichia coli</i>	NBRC 15034	Clinical specimen	+	+		
<i>Escherichia coli</i>	NBRC 102203T	urine	+	+		
<i>Escherichia coli</i>	NBRC 13500	Unknown	+	+		
<i>Escherichia coli</i>	NBRC 15034	Clinical specimen	+	+		
<i>Escherichia coli</i>	ATCC 25922	Unknown	+	+		
<i>Escherichia coli</i>	NBRC 13966	unknown	+	+		
<i>Escherichia coli</i>	NBRC 13898	Unknown	+	+		
<i>Escherichia coli</i>	NBRC 3301	unknown	+	+		
<i>Escherichia coli</i>	NBRC 3302	Unknown	+	+		
<i>Escherichia coli</i>	NBRC 13540	unknown	+	+		
<i>Escherichia coli</i>	NBRC 3366	Unknown	+	-		
<i>Escherichia coli</i>	NBRC 3543	unknown	+	+		
<i>Escherichia coli</i>	NBRC 3544	Unknown	+	+		
<i>Escherichia coli</i>	NBRC 14129	unknown	+	+		
<i>Escherichia coli</i>	NBRC 15484	Unknown	+	+		
<i>Escherichia coli</i>	NBRC 12062	unknown	+	+		
<i>Escherichia coli</i>	NBRC 12433	Unknown	+	+		
<i>Escherichia coli</i>	NBRC 12734	unknown	+	+		
<i>Escherichia coli</i>	NBRC 3972	Feces	+	+		
<i>Escherichia coli</i>	NBRC 3991	unknown	+	+		
<i>Escherichia coli</i>	NBRC 13891	Unknown	+	-		
<i>Escherichia coli</i>	NBRC 13892	unknown	+	-		
<i>Escherichia coli</i>	NBRC 3545	Unknown	+	+		
<i>Escherichia coli</i>	NBRC 3546	unknown	+	+		
<i>Escherichia coli</i>	NBRC 3806	Unknown	+	+		
<i>Escherichia coli</i>	NBRC 3993	Unknown	+	-		
<i>Escherichia fergusonii</i>	NBRC 102419	Feces of human (one-year-old boy)	+	+		
<i>Escherichia hermanii</i>	NBRC 105704T	Toe of 17-year-old female	+	+		
<i>Escherichia vulneris</i>	NBRC 102420	Human wound	+	+		

<i>Klebsiella oxytota</i>	NBRC 105695	Pharyngeal tonsil	+	+
<i>Klebsiella pneumoniae</i>	NBRC 14940T	Unknown	+	+
<i>Klebsiella pneumoniae</i>	ATCC 13883	Unknown	+	+
<i>Klebsiella pneumoniae</i>	Natural isolate #31	Raw yellowtail	+	+
<i>Kluyvera cryocrescens</i>	Natural isolate #2	food	+	+
<i>Kluyvera intermedia</i>	NBRC 102594T	Surface water	+	+
<i>Leclercia adecarboxylata</i>	NBRC 102595	Drinking water	+	+
<i>Pantoea agglomerans</i>	Natural isolate #3	Cake	+	+
<i>Rahnella aquatilis</i>	Natural isolate #10	Raw ground pork	+	+
<i>Raoultella planticola</i>	NBRC 14939	Radish root	+	+
<i>Raoultella terrigena</i>	Natural isolate #33	Raw salmon	+	+
<i>Raoultella terrigena</i>	NBRC 14941T	Drinking water	+	+

Strain Name	Source	Origin	Results	
			Medi-Ca CC	BGLB
<i>Achromobacter denitrificans</i>	NBRC 15125T	soil	-	-
<i>Achromobacter xylosoxidans</i>	NBRC 15126	Ear discharge	-	-
<i>Aeromonas hydrophila</i>	NBRC 12658	unknown	-	-
<i>Alcaligenes faecalis</i>	NBRC 13111T	Unknown	-	-
<i>Bacillus amyloliquefaciens</i>	Natural isolate #8	Powdered paprika	-	-
<i>Bacillus cereus</i>	NBRC 15305T	Unknown	-	-
<i>Bacillus cereus</i>	NBRC 3836	Unknown	-	-
<i>Bacillus cereus</i>	NBRC 13494	Unknown	-	-
<i>Bacillus licheniformis</i>	Natural isolate #4	Cheese cake	-	-
<i>Bacillus subtilis</i>	Natural isolate #14	Chinese barbecued pork	-	-
<i>Bacillus subtilis</i>	NBRC 3134	Unknown	-	-
<i>Bacillus subtilis</i>	NBRC 15286	Food	-	-
<i>Corynebacterium variabile</i>	NBRC 105688T	Human feces	-	-
<i>Edwardsiella tarda</i>	Natural isolate #5	Cheese cake	-	-
<i>Kocuria kristinae</i>	NBRC 3202	Sour grain mash	-	-
<i>Lactobacillus delbrueckii</i>	Natural isolate #40	Yoghurt	-	-
<i>Lactococcus lactis</i>	Natural isolate #42	Lactic acid drink	-	-
<i>Lactobacillus casei</i>	NBRC 3333T	Unknown	-	-
<i>Micrococcus luteus</i>	NBRC 13867	Air	-	-
<i>Micrococcus luteus</i>	NBRC 15355T	Human skin	-	-
<i>Micrococcus lyliae</i>	NBRC 3851	Unknown	-	-
<i>Proteus hauseri</i>	NBRC 105696	Unknown	-	-
<i>Proteus hauseri</i>	NBRC 105697T	Unknown	-	-
<i>Proteus mirabilis</i>	NBRC 105687T	Feces	-	-
<i>Providencia alcalifaciens</i>	NBRC 14162	Soil enrichment with ethanol as carbon source	-	-
<i>Pseudomonas mendocina</i>	NBRC 3453	Unknown	-	-
<i>Pseudomonas aeruginosa</i>	NBRC 12689	Unknown	-	-
<i>Pseudomonas aeruginosa</i>	ATCC 9027	Unknown	-	-
<i>Pseudomonas aeruginosa</i>	NBRC 3446	Urine	-	-
<i>Pseudomonas aeruginosa</i>	NBRC 3449	Urine	-	-
<i>Pseudomonas fluorescens</i>	Natural isolate #16	Raw lamb	-	-
<i>Pseudomonas fluorescens</i>	Natural isolate #22	White radish sprouts	-	-
<i>Pseudomonas pseudoalgenes</i>	NBRC 14167	Sinus drainage	-	-
<i>Pseudomonas stutzeri</i>	NBRC 14165	Human spinal fluid	-	-
<i>Serratia liquefaciens</i>	Natural isolate #12	Raw ground chicken	-	-
<i>Serratia marcescens</i>	NBRC 102204	Pond water	+	-
<i>Staphylococcus aureus</i>	ATCC 33862	Unknown	-	-
<i>Staphylococcus aureus</i>	NBRC 14462	Clinical isolate	-	-
<i>Staphylococcus aureus</i>	NBRC 100910T	Human pleural fluid	-	-
<i>Staphylococcus aureus</i>	NBRC 12732	Human lesion	-	-
<i>Streptococcus equines</i>	NBRC 12553T	Unknown	-	-
<i>Streptococcus thermophilus</i>	Natural isolate #41	Yoghurt	-	-

^aThe natural isolate strains were isolated and numbered in our laboratory, and then identified by molecular and biochemical analyses.

^b+ = detected; - = detected

Table 2. Matrix Study Results (Method Developer) (1)

Matrix	Inoculation Microorganism	Contamination Level	Media CC			VRBA				p-value	Mean Difference	95% CI ^d LCL ^e UCL ^f		
			Mean ^a	s ^b	RSD _r ^c	Mean _r	s _r	RSD _r	r ^{2g}					
Raw ground pork	N/A ^h	Low	4.19	0.03	0.71	4.18	0.06	1.36	0.69	-0.01	-	0.06		
		Medium	5.06	0.04	0.80	5.09	0.02	0.48	0.11	0.03	-	0.07	1.00	
		High	8.42	0.06	0.69	8.47	0.11	1.30	0.53	0.23	-	0.25		
Raw lamb	N/A	Low	2.31 ⁱ	0.09	3.83	2.48	0.05	1.95	0.01	0.18	0.	0.27		
		Medium	7.79	0.04	0.48	7.82	0.03	0.33	0.25	0.02	-	0.07	1.00	
		High	8.59 ⁱ	0.04	0.46	8.69	0.07	0.86	0.04	0.09	0.	0.18		
Raw ground chicken	N/A	Low	2.11 ⁱ	0.07	3.47	2.22	0.09	4.20	0.08	0.11	-	0.23		
		Medium	3.62 ⁱ	0.08	2.08	3.75	0.09	2.35	0.05	0.13	0.	0.26	0.99	
		High	5.02	0.02	0.43	4.93	0.12	2.43	0.14	-0.09	-	0.04		
Raw tuna fillet	N/A	Low	2.53	0.06	2.23	2.54	0.05	1.85	0.76	0.01	-	0.13		
		Medium	3.56	0.10	2.89	3.42	0.10	2.98	0.06	-0.13	-	0.01	1.00	
		High	6.41	0.03	0.52	6.27	0.13	2.12	0.09	-0.13	-	0.03		
Raw salmon fillet	N/A	Low	2.16	0.19	8.99	2.02	0.08	4.06	0.25	-0.15	-	0.15		
		Medium	3.04	0.02	0.79	3.06	0.06	1.84	0.65	0.01	-	0.08	0.97	
		High	3.97	0.03	0.71	3.96	0.06	1.45	0.61	-0.01	-	0.00	0.	
Raw shrimp	N/A	Low	2.42 ⁱ	0.08	3.41	2.16	0.17	7.66	0.01	-0.26	-	-0.10		
		Medium	3.53	0.06	1.81	3.47	0.06	1.70	0.24	-0.06	-	0.06	1.00	
		High	8.41 ⁱ	0.03	0.34	8.23	0.03	0.42	0.00	-0.18	-	-0.11		
Fresh peeled banana	E.coli NBRC 15034	Uninoculated	<1.00	—	—	<1.00	—	—	—	—	—	—	—	—
		Low	3.51	0.11	3.28	3.49	0.06	1.74	0.73	-0.02	-	0.12		
		Medium	4.75	0.09	1.86	4.76	0.09	1.84	0.88	0.01	-	0.19	0.99	
		High	5.65	0.03	0.58	5.68	0.07	1.24	0.40	0.03	-	0.13		
Fresh cut pineapple	ATCC 25922	Uninoculated	<1.00	—	—	<1.00	—	—	—	—	—	—	—	—
		i		0.68	3.34	0.03	0.93	0.01	0.06	0.	0.10			
		Medium	4.41	0.04	0.95	4.41	0.04	0.94	0.94	0.00	-	0.06	1.00	
		High	5.42	0.03	0.57	5.40	0.03	0.49	0.33	-0.02	-	0.03		
Fresh cut apple	E. aerogenes NBRC 13534	Uninoculated	<1.00	—	—	<1.00	—	—	—	—	—	—	—	—
		Low	3.60	0.06	1.54	3.58	0.05	1.46	0.34	-0.02	-	0.03		
		Medium	4.67	0.08	1.71	4.64	0.11	2.38	0.70	-0.03	-	0.19	1.00	
		High	5.74	0.04	0.63	5.65	0.08	1.46	0.11	-0.09	-	0.03		

^a Mean of 5 replicates after the logarithmic transformation: $\log_{10}[\text{CFU/g} + (1.0)]$.^b RSD_r = standard deviation.^c RSD_r = relative standard deviation.^d CI = confidence interval.^e LCL = lower confidence limit.^f UCL = upper confidence limit.^g r² = square of the correlation coefficient.^h N/A = Not applicable. Samples are naturally contaminated.ⁱ Significantly different ($p < 0.05$).

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